

CCV. THE CARBOHYDRATE AND FAT METABOLISM OF YEAST

V. THE SYNTHESIS OF FAT FROM ACETIC ACID: THE INFLUENCE OF METALLIC IONS ON CARBOHYDRATE AND FAT STORAGE

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WHEN yeast is suspended in oxygenated water, part of its reserve carbohydrate is transformed into lipid; if, however, it be incubated in an oxygenated solution of sodium acetate, the amount of lipid formed is greater and is indubitably formed at the expense of the acetate molecule [Smedley-Maclean & Hoffert, 1926; Wieland & Wille, 1935].

There appeared to be three possible ways in which this synthesis could be effected: (1) the fatty acid chains might be built up at the expense of the acetate molecules; (2) the reserve carbohydrate present in the cell might furnish some degradation product capable of reacting with the acetate molecules; (3) carbohydrate might be formed from the acetate and converted into fat.

It was therefore interesting to know whether any simple carbon compound formed by the condensation of acetic acid could be utilized for fat formation. Butyric, acetoacetic and β -hydroxybutyric acids had already been tested with negative results [Smedley-Maclean & Hoffert, 1926]; succinic acid, formed in a yield of 10% when yeast is incubated in an oxygenated acetate solution and citric acid, formed from acetate in presence of Ba^{++} ions [Wieland & Sonderrhoff, 1932], have now been tested and found to be ineffective as are also fumaric and malic acids. Acetoin, synthesized by yeast in large yields when acetaldehyde is added to a fermenting sugar solution [Neuberg & Reinfurth, 1923], was also inactive. The results of our experiments are set forth in Table I.

Yeast incubated in the solutions tested showed about the same fat content as yeast which has been incubated in oxygenated water, with three exceptions, diacetyl, methylvinyl ketone and sodium sorbate, in all of which a system of conjugated double bonds is present; this structure apparently inhibits the transformation of reserve carbohydrate into fat normally taking place when yeast is incubated in oxygenated water.

So far, therefore, it has not been possible to find evidence that any simple carbon compound containing 4-6 carbon atoms, which might be regarded as a condensation product of acetic acid, can act as an intermediate substance in the synthesis of fat.

In support of the hypothesis that fatty acids might be formed by the interaction of the acetate molecule with some degradation product of carbohydrate, experiments carried out by Stephenson & Whetham [1922] were recalled in which the addition of sodium acetate to the glucose solution in which the Timothy grass bacillus was incubated resulted in an increase of the amount of fat stored.

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Table I

Showing mg. of fat and carbohydrate contained in 10 g. yeast after incubation in an oxygenated solution of substance to be tested. The figures after incubation of 10 g. of the same sample of yeast in oxygenated Na acetate solution are given for comparison. The phosphate present in the solution was 1.27% as a mixture of sodium hydrogen phosphates pH 6.8. CH = carbohydrate; Lipoid = ether-soluble material. The methods of estimation were those used by Smedley-Maclean & Hoffert.

| Substance | % | Yeast after incubation in | | | | | | | |
|---|------|---------------------------|-----|------------------------------|--------|------------------------|--------|---|--------|
| | | Original yeast | | (1) Solution Na acetate N/14 | | (2) Solution substance | | (3) Solution substance with $\text{PO}_4^{=}$ | |
| | | | | CH | Lipoid | CH | Lipoid | CH | Lipoid |
| Na salt of: | | | | | | | | | |
| Citric acid | 0.52 | — | 84 | 237 | 190 | 250 | 117 | — | — |
| Succinic acid | 0.44 | — | 86 | 250 | 214 | 377 | 134 | 310 | 153 |
| | 0.22 | 760 | 82 | 165 | 123 | 310 | 137 | 250 | 144 |
| Fumaric acid | 0.43 | 595 | 83 | 232 | 195 | — | — | 165 | 153 |
| Maleic acid | 0.43 | — | — | — | — | — | — | 200 | 145 |
| Crotonic acid | 0.32 | — | — | 302 | 203 | 245 | 109 | — | — |
| Laevulic acid | 1.00 | 560 | 86 | 185 | 207 | — | — | 195 | 128 |
| Acetoin | 0.32 | 637 | 84 | 237 | 190 | — | — | 167 | 134 |
| | 0.50 | 842 | 89 | 167 | 242 | — | — | 170 | 213 |
| | 0.50 | 750 | 104 | 180 | 234 | — | — | 192 | 173 |
| | 0.50 | 742 | 84 | 230 | 220 | — | — | 222 | 175 |
| | 0.50 | 645 | 80 | 192 | 198 | — | — | 192 | 141 |
| | 0.64 | 850 | 89 | 325 | 230 | 280 | 143 | 192 | 157 |
| | 0.78 | 742 | 82 | 345 | 185 | — | — | 197 | 151 |
| 2:3-Butylene glycol | 0.66 | 820 | 87 | 192 | 230 | 415 | 128 | 295 | 153 |
| Methylethyl ketone | 0.30 | — | 80 | — | 173 | — | — | — | 137 |
| Substances containing conjugated double bonds | | | | | | | | | |
| Diacetyl | 0.65 | 750 | 91 | 185 | 196 | 645 | 84 | 385 | 81 |
| Methylvinyl ketone | 0.40 | 750 | 104 | 180 | 234 | — | — | 267 | 96 |
| | 0.40 | 742 | 84 | 230 | 220 | — | — | 360 | 77 |
| Sodium sorbate | 1.00 | 712 | 87 | 187 | 243 | 292 | 74 | 150 | 87 |
| | 1.00 | 802 | 87 | 212 | 201 | 337 | 74 | 187 | 98 |

The effect of adding sodium acetate to a pure glucose solution in which yeast was incubated was to produce no increase in lipid but to bring about a considerable lowering of the carbohydrate content.

In Stephenson & Whetham's experiments the medium in which the Timothy grass bacillus was grown contained glucose, CaCO_3 to neutralize any free acids produced, and inorganic salts, ammonium salts furnishing a supply of nitrogen. As we were concerned only with the transformation of glucose into lipid in the existing cells and desired to exclude the formation of new cells a nitrogen supply had to be eliminated but we proceeded to examine the effect of the addition of CaCO_3 to the glucose medium in which the yeast was incubated.

When CaCO_3 was added to the glucose solution in which the yeast was incubated the amount of lipid fell to about two-thirds of that formed in the absence of CaCO_3 . Addition of sodium acetate to the glucose and CaCO_3 medium produced, however, a slight rise in the amount of lipid stored (cp. Table II); the carbohydrate content showed no significant alteration.

The addition of CaCO_3 to the glucose solution produced a distinct sedimentation of the yeast, and it seemed possible that this mechanical effect might have interfered with the formation of lipid for which free oxygenation is essential. The replacement of the CaCO_3 by kieselguhr both in its original state and after washing with HCl produced fairly rapid settling of the yeast and in its

Table II

Showing the effect of CaCO_3 on fat and carbohydrate storage by yeast incubated in oxygenated solutions of glucose and of glucose and sodium acetate respectively. CH = carbohydrate. Figures represent mg./10 g. yeast

| Original | | Glucose | | Glucose + CaCO_3 | | Glucose + Na acetate | | Glucose + CaCO_3 + Na acetate | |
|----------|-----|---------|-----|---------------------------|-----|----------------------|-----|--|-----|
| Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH |
| 78 | 655 | — | — | 216 | 667 | — | — | 253 | 640 |
| 115 | 460 | 452 | 540 | 315 | 610 | — | — | 359 | 720 |
| 80 | — | 406 | — | — | — | 381 | — | — | — |
| 98 | 700 | 415 | 550 | — | — | 415 | 400 | — | — |
| 79 | 550 | 415 | 600 | — | — | 408 | 350 | — | — |
| 89 | 720 | 403 | 660 | — | — | 335 | 470 | — | — |
| 74 | 400 | 326 | 560 | 223 | 520 | — | — | 231 | 450 |
| Mean: 88 | 550 | 402 | 580 | 251 | 600 | 385 | 370 | 281 | 600 |

original state a fall of stored lipid similar to that produced by CaCO_3 . After the kieselguhr had been well washed with acid, no inhibitory effect was produced on the lipid formation: the washings contained Ca^{++} and Mg^{++} removed from the original kieselguhr. These results suggested that Ca^{++} or Mg^{++} and not the sedimentation was the inhibiting factor.

Table III

Showing mg. lipid and carbohydrate stored in 10 g. yeast after incubation in oxygenated 2% glucose solution to which kieselguhr had been added.

| Original | | After incubation in | | | | | |
|---------------------|-----|-------------------------|-----|---|-----|--|-----|
| | | (a) 2% glucose solution | | (b) 2% glucose solution with kieselguhr | | (c) 2% glucose solution with N/14 Na acetate | |
| | | Lipoid | CH | Lipoid | CH | Lipoid | CH |
| (1) Unwashed | | | | | | | |
| 89 | 670 | 309 | 745 | 239 | 862 | 305 | — |
| 96 | 672 | 392 | 580 | 282 | 560 | 396 | 457 |
| (2) Washed with HCl | | | | | | | |
| 88 | 680 | 371 | 652 | 345 | 565 | 313 | 600 |
| 80 | 642 | 415 | 457 | 400 | 565 | 391 | 402 |

Table IV

Showing mg. lipid in 10 g. yeast after suspension in oxygenated solutions of 2% glucose to which had been added (b) CaCO_3 , (c) sodium acetate + CaCO_3 , (d) sodium glycollate + CaCO_3 , (e) sodium succinate + CaCO_3 .

| Original yeast Lipoid | (a) Glucose Lipoid | (b) Glucose + CaCO_3 Lipoid | (c) Glucose, CaCO_3 and N/14 Na acetate Lipoid | (d) Glucose, CaCO_3 and N/14 Na glycollate Lipoid | (e) Glucose, CaCO_3 and N/14 Na succinate Lipoid |
|-----------------------|--------------------|--------------------------------------|---|--|---|
| 102 | — | 227 | — | 381 | — |
| 79 | — | 329 | — | 397 | — |
| 78 | — | 216 | 253 | 276 | — |
| 84 | — | 195 | — | 256 | 278 |
| 115 | 452 | 315 | 359 | — | — |
| 74 | 326 | 223 | 231 | — | — |

The increase of lipid produced by the addition of sodium acetate to the glucose- CaCO_3 medium was not specific for the acetate but was produced by the addition of other organic Na salts to the medium, and probably indicated an antagonistic effect of the Na^+ and Ca^{++} ions.

The inhibitory effects produced on lipid storage by the addition of NaCl , CaCl_2 and MgCl_2 respectively to the glucose medium are set forth in Table V.

Table V

Showing the lipid and carbohydrate (CH) content in mg. per 10 g. yeast after incubation in 2% glucose solution with and without the addition of chlorides. Medium oxygenated for 24 hr.

| Original yeast | | Medium | | | | | | | | | | | |
|----------------|-----|----------------|-----|-----------------------|--------|-----|------------------------------------|--------|-----|------------------------------------|--------|-----|--|
| | | (a) 2% glucose | | (b) 2% glucose + NaCl | | | (c) 2% glucose + CaCl ₂ | | | (d) 2% glucose + MgCl ₂ | | | |
| | | Lipoid | CH | NaCl | Lipoid | CH | CaCl ₂ | Lipoid | CH | MgCl ₂ | Lipoid | CH | |
| 100 | 590 | 404 | 580 | N/14 | 400 | 420 | N/14 | 272 | 500 | N/14 | 261 | 580 | |
| 100 | 380 | 392 | 470 | N/14 | 406 | 340 | N/14 | 242 | 330 | N/14 | 244 | 370 | |
| 92 | 660 | 443 | 747 | — | — | — | N/350 | 280 | 727 | — | — | — | |
| 95 | 610 | 401 | 660 | — | — | — | — | — | — | — | — | — | |
| — | 740 | 429 | 630 | — | — | — | N/350 | 275 | 640 | N/14 | 277 | 410 | |
| | | | | | | | N/700 | 333 | 700 | N/140 | 298 | 550 | |
| | | | | | | | N/140 | 242 | 630 | N/1400 | 371 | 580 | |
| 98 | 740 | 432 | 610 | — | — | — | N/14 | 228 | 450 | — | — | — | |
| | | | | | | | N/140 | 232 | 600 | — | — | — | |
| | | | | | | | N/1400 | 358 | — | — | — | — | |

The $p\text{H}$ of the medium at the end of these experiments was in each case approximately between 3 and 5. The variation in $p\text{H}$ seemed to have little effect on the power of lipid synthesis.

These experiments brought home to us the fact that the nature of the metallic ions present in the medium exercised a considerable influence on the lipid storage. Further investigation of the comparative effects of the ions, K^+ , Na^+ , Ca^{++} and Mg^{++} , confirmed that both Ca^{++} and Mg^{++} exercise a very marked inhibition on the formation of lipid material from glucose, Na^+ being almost without action; on the other hand, the presence of Na^+ , Ca^{++} or Mg^{++} reduced, to some extent, the carbohydrate content.

An instance of the marked inhibitory effect of Ca^{++} and Mg^{++} was provided by some experiments in which yeast was incubated in media consisting respectively of glucose and commercial fructose solutions. The yeast incubated in the fructose solution had a very low lipid content which showed no diminution when CaCO_3 was added to the medium and was much lower than the values previously recorded in similar experiments [Smedley-Maclean & Hoffert, 1923]. The sample of commercial fructose gave a good reaction for Ca and Mg but the purified fructose behaved similarly to the glucose.

The effects of K^+ , Na^+ , Ca^{++} and Mg^{++} , were then compared by incubating yeast in glucose solutions to which their acetates had been separately added. The results are shown in Table VI.

Here also little effect was produced on the amount of fat stored by the presence of K^+ and Na^+ , but Ca^{++} and Mg^{++} produced marked diminutions, Mg^{++} apparently having the greatest influence. The inhibitory effect on lipid storage appeared to be greater in the presence of the chlorides than of the acetates, a result consistent with the view that the inhibition brought about by Ca^{++} and Mg^{++} might be to some extent compensated by the additional lipid formed from the acetate ions.

Table VI

Showing mg. lipid and carbohydrate (CH) in 10 g. yeast after incubation for 24 hr. in an oxygenated solution of 2% glucose with *N*/14 acetate (0.72%).

| Original yeast | | Glucose 2% | | Glucose 2% + K acetate | | Glucose 2% + Na acetate | | Glucose 2% + Ca acetate | | Glucose 2% + Mg acetate | |
|----------------|-----|------------|-----|---------------------------|-----|----------------------------|-----|----------------------------|-----|----------------------------|-----|
| Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH |
| 80 | 710 | 398 | 585 | — | — | 381 | 395 | 311 | — | — | — |
| 82 | 760 | 370 | 730 | — | — | — | — | 317 | 550 | 287 | 780 |
| 79 | 550 | 415 | 600 | — | — | 408 | 350 | 342 | 460 | 309 | 380 |
| 89 | 722 | 403 | 660 | — | — | 335 | 470 | 309 | 520 | 289 | 570 |
| 98 | 700 | 415 | 550 | — | — | 415 | 400 | 402 | 370 | 325 | 370 |
| 97 | 530 | — | 550 | — | — | — | — | 297 | 440 | — | — |
| 91 | — | 436 | — | — | — | 422 | — | 388 | — | 399 | — |
| 82 | 420 | 364 | 560 | 392 | 670 | 374 | 440 | — | — | 306 | 537 |

The carbohydrate content of the yeast was also less in the presence of Na^+ , Ca^{++} and Mg^{++} , Na^+ being on the whole the most effective inhibitor of the three. Estimations of the residual sugar showed that in the presence of Ca^{++} the amount of residual sugar was higher, the utilization of the sugar having been less effective; Na^+ and Mg^{++} had no perceptible effect.

When yeast was incubated in oxygenated solutions of the pure acetates, the influence of the ions was closely similar to that produced in the glucose-acetate solutions. The presence of K^+ was most favourable for the storage of both lipid and carbohydrate; unlike Mg^{++} , Na^+ left the lipid content almost unaffected but like Mg^{++} it produced a decrease in the amount of carbohydrate; Ca^{++} caused a marked lowering of both lipid and carbohydrate contents.

Incubation of yeast in oxygenated solutions of acetates (N/14)

Table VII

Showing mg. lipid and carbohydrate stored in 10 g. yeast after 24 hr. incubation in acetate solutions, *N*/14.

| Original yeast | | After incubation in | | | | | | | | | |
|----------------|-----|---------------------|-----|---------------|-----|----------------|-----|----------------|-----|----------------|-----|
| | | (1) Water | | (2) K acetate | | (3) Na acetate | | (4) Ca acetate | | (5) Mg acetate | |
| Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH | Lipoid | CH |
| 87 | 700 | — | — | 227 | 343 | 230 | 248 | — | — | 185 | 262 |
| 89 | 843 | — | — | 266 | 263 | 242 | 168 | — | — | — | — |
| 92 | 825 | — | — | 266 | 232 | 245 | 230 | — | — | — | — |
| 95 | 523 | — | — | 231 | 260 | 198 | 180 | — | — | — | — |
| 85 | 650 | — | — | — | — | 213 | 240 | 170 | 250 | — | — |
| 87 | 620 | 139 | 220 | — | — | 237 | 280 | 150 | 180 | 191 | 280 |
| 93 | 700 | 134 | 230 | — | — | 225 | 260 | 206 | 230 | 199 | 280 |
| 87 | 790 | 125 | 300 | — | — | 241 | 340 | 185 | 220 | 193 | 300 |
| 89 | 740 | 128 | 340 | — | — | 257 | 350 | 166 | 250 | — | — |
| 89 | 670 | 111 | 300 | — | — | 240 | 320 | 181 | 240 | — | — |

The effect of increasing the concentration of acetate in the medium on the storage products was determined in solutions of the K salt. No further increase was obtained by raising the percentage of acetate from 0.29 to 1.15%.

The effects of the ions upon yeast with low and high reserves of carbohydrate were then compared. Samples of a yeast with a high carbohydrate content were suspended for 24 hr. in oxygenated water, and in oxygenated solutions of Ca and Na acetates respectively. The yeast from the oxygenated water was then

Table VIIA

Showing lipid and carbohydrate contents in mg./10 g. yeast after incubation in K acetate solution.

| | Lipoid | CH |
|-----------------------------|--------|-----|
| Original yeast | 92 | 530 |
| Yeast after incubation in: | | |
| 0.03% acetic acid as K salt | 122 | 250 |
| 0.06% " | 158 | 290 |
| 0.29% " | 209 | 340 |
| 0.58% " | 219 | 310 |
| 1.15% " | 191 | 320 |
| | 208 | 270 |

transferred to the acetate solutions and the effects on carbohydrate and fat storage compared with those in the yeast which had been at once placed in the acetate solutions.

Table VIII

Showing lipid content in mg. when 10 g. original yeast from brewery were incubated in oxygenated acetate solutions before and after suspension in oxygenated water. ("Run-down" yeast = yeast after suspension in water.)

| | Yeast before incubation in acetate solution | | Yeast after incubation in | | | |
|------------|--|-----|---------------------------|-----|---------------------|-----|
| | | | Na acetate solution | | Ca acetate solution | |
| Exp. I | Lipoid | CH | Lipoid | CH | Lipoid | CH |
| Original | 89 | 735 | 257 | 350 | 166 | 255 |
| Run down | 127 | 480 | 261 | 320 | 215 | 280 |
| Difference | +38 | | +4 | | +49 | |
| Exp. II | | | | | | |
| Original | 89 | 670 | 240 | 317 | 181 | 245 |
| Run down | 111 | 432 | 236 | 305 | 200 | 265 |
| Difference | +22 | | -4 | | +19 | |

The yeast made substantially the same amount of fat whether it was at once incubated in the Na acetate solution or whether it was first incubated in oxygenated water, and then transferred to the Na acetate solution. On the other hand, the yeast which had been at once added to the Ca acetate solution had a lower fat content than the yeast transferred to the Ca acetate solution after incubation in oxygenated water. A possible explanation was that the Ca, whilst reducing the amount of lipid formed from acetate, almost entirely inhibited the formation of fat from the reserve carbohydrate. This was, however, not confirmed since yeast incubated in solutions of CaCl_2 , with and without CaCO_3 , formed fat just as well as when incubated in oxygenated water. Possibly some product formed from the Ca acetate exercised an inhibitory effect.

Estimation of the residual acetate in the media. Determination of the acetate in the residual media showed that more acetate had disappeared from the Ca (30%) than from the Na acetate solution (19.5%). In both cases about twice as much acetate disappeared when the yeast used had already been incubated in water and contained a low carbohydrate reserve (Na acetate, 47.6%; Ca acetate, 74.3%).

The effect of phosphate on lipid formation from acetate. The mean lipid content in 8 experiments in which 10 g. yeast were incubated in 0.6% Na acetate solution was 0.218 g.; in 16 experiments in which yeast was incubated in 0.6% Na acetate solution containing 1.27% phosphate (as mixed sodium

hydrogen phosphates $pH=6.8$) the mean lipid content was 0.222 g. The addition of phosphate to the acetate solution therefore produced no significant result.

The composition of the lipid substance collected from a series of parallel experiments in which samples of the same yeast had been incubated in the various media, is shown in Table IX.

Table IX

| Medium | Lipoid g. | Saponifi- cation value | Unsaponifiable | | Fatty acids | |
|-------------------------|--------------|------------------------------|----------------|------|-------------|------|
| | | | Wt. g. | % | Wt. g. | % |
| (a) Original yeast | 1.364 | 148.8 | 0.464 | 34.0 | 0.860 | 63.0 |
| 2% glucose | 2.319 | 160.4 | 0.595 | 25.6 | 1.588 | 68.5 |
| 0.6% Na acetate | 1.757 | 160.4 | 0.453 | 25.8 | 1.292 | 73.5 |
| (b) Glucose | 1.277 | 180.7 | 0.236 | 18.5 | 0.946 | 74.1 |
| Glucose with Na acetate | 1.206 | 208.9 | 0.119 | 9.9 | 0.921 | 76.4 |
| Glucose with Mg acetate | 0.963 | 217.8 | 0.087 | 9.6 | 0.768 | 79.7 |
| Glucose with Ca acetate | 1.092 | 208.7 | 0.099 | 9.1 | 0.880 | 80.6 |
| (c) Glucose | 0.727 | 168.0 | 0.198 | 27.3 | 0.501 | 68.9 |
| Glucose with K acetate | 0.784 | 155.8 | 0.226 | 28.7 | 0.530 | 67.6 |
| Glucose with Na acetate | 0.748 | 161.4 | 0.198 | 27.1 | 0.530 | 70.8 |
| Glucose with Mg acetate | 0.612 | 172.9 | 0.166 | 26.4 | 0.432 | 70.6 |

In Exp. (b) the addition of the acetate to the glucose medium produced a fall in the unsaponifiable from 18.9 to between 9 and 10%; this result was not confirmed in other experiments. The addition of Na^+ , Mg^{++} and Ca^{++} showed, however, a tendency to raise the proportion of fatty acids to unsaponifiable matter.

Detection of pyruvic acid and of acetaldehyde in the residual media on addition of Na^+ , Ca^{++} and Mg^{++} to the glucose solution

When 2:4-dinitrophenylhydrazine reagent was added to the residual glucose and glucose-acetate solutions, a marked difference was observed in the amounts of precipitate which separated immediately at ordinary temperature.

Whereas the residual glucose solution generally remained clear or showed only a slight cloudiness, the glucose solutions to which K, Na, Ca or Mg acetates had been added gave quite copious precipitates, usually most marked in the case of the glucose-Mg acetate medium. Replacement of the acetate solutions by chlorides produced somewhat similar precipitates and the accumulation of carbonyl compounds thus appears to be promoted by the presence of K^+ , Na^+ , Ca^{++} and Mg^{++} .

Quantitative experiments were carried out in which 40 g. of yeast were incubated in 2 l. of an oxygenated 2% glucose solution to which Na, Ca and Mg acetates were respectively added ($N/14$). To the resulting filtrates excess of 2:4-dinitrophenylhydrazine reagent was added and the resulting precipitates, formed after 10 min. stirring, filtered, dried at 37° and weighed. These were then extracted with Na_2CO_3 solution and the weights of acid and neutral fractions determined.

| | 2% glucose | 2% glucose + Na acetate | 2% glucose + Ca acetate | 2% glucose + Mg acetate |
|----------------------|------------|----------------------------|----------------------------|----------------------------|
| Wt. total ppt. g. | 0.22 | 1.93 | 1.82 | 1.71 |
| Wt. neutral fraction | 0.17 | 1.45 | 0.95 | 0.90 |
| Wt. acid fraction | 0.05* | 0.43 | 0.83 | 0.71 |

* By difference.

The greater part of the neutral fraction consisted of acetaldehyde dinitro phenylhydrazone; the acid fraction consisted mainly of the pyruvic acid derivative identified by the melting point of its ethyl ester.

A number of comparative experiments were carried out in which portions of 1 g. yeast were incubated in oxygenated glucose solutions to which were added the acetate or chloride of K, Na, Ca or Mg; in some cases Mg or Ca carbonate was added to the respective chloride solution to prevent the development of acidity. The results showed considerable variation but the glucose medium to which K, Mg, Ca or Na acetate or chloride had been added always gave a precipitate with the hydrazine reagent. The glucose-acetate (K, Na and Mg) solutions, when tested with sodium nitroprusside, acetic acid and ammonia, usually developed a blue colour showing the presence of pyruvic acid in the solution. The precipitates and pyruvic acid reaction were only marked in media which had been well oxygenated. The glucose-chloride solutions, like the glucose solution, were strongly acid in contrast to the glucose-acetate solutions, the *pH* of which was usually 5–6.

DISCUSSION OF RESULTS

Influence of metallic ions on fat and carbohydrate metabolism

Evidence already exists that the nature of the ions in the medium exercises an important influence on the fermentation process. Harden [1917] showed that washed zymin was readily activated by acetaldehyde in the presence of K but not of Na phosphate. When glucose is fermented with yeast juice, the rate of fermentation is delayed if the concentration of sodium phosphate exceeds a certain minimum. Meyerhof [1918] pointed out that NaCl and other salts depress both the rate of attainment of the maximum rate of fermentation and the maximum rate attained; Harden & Henley [1921] found that the chlorides and sulphates of Na and K diminished the maximum rate of fermentation of glucose and fructose by zymin and also the rate of attainment of this maximum, an effect not appreciably modified by the addition of acetaldehyde. The rate of decomposition of pyruvic acid was, however, uninfluenced. An inhibitory action of Na^+ on the phosphorylating coenzymes is described in recent work by Ohlmeyer & Ochoa [1937]; the activities of cozymase and adenylic acid as phosphorylating agents in a medium containing Na_2HPO_4 were 1 : 60, but after the addition of a trace of Mn^{++} to the medium the activities of both increased to 200 : 300. Cozymase was much more sensitive to the inhibitory action of Na^+ than was adenylic acid; the ratio of the concentrations of Mn^{++} , Mg^{++} , K^+ and NH_4^+ , which removed the inhibitory influence of the Na^+ , was 1 : 50 : 1000 : 2000. Our knowledge of the inhibitory action of Ca^{++} seems to rest chiefly on the work of Fernbach & Schoen [1913; 1914; 1920] who found that considerable quantities of pyruvic acid could be isolated from glucose fermented by various yeasts, notably *Mycovelvure Duclaux* in the presence of CaCO_3 . They ascribed the effect not only to the reaction of the medium, since when beer wort was used as the medium no pyruvic acid could be isolated even after the addition of chalk.

In Neuberg's laboratory, pyruvic acid was isolated from the action of yeast juice on Mg hexosediphosphate solution, glycerol being also formed [Kobel & Scheuer, 1930]. The addition of MgHPO_4 , MgO or Na_2HPO_4 to a 10% glucose solution in which fresh yeast was fermenting produced similar results. Neuberg & Kobel [1930] concluded that the phosphate ions were not concerned since MgO produced the same effect, and that Mg ions were not essential since Na_2HPO_4 produced a similar result.

The influence of metallic ions on the storage of fat and carbohydrate by yeast in oxygenated glucose media

In our experiments the addition of CaCl_2 or MgCl_2 to the oxygenated glucose solution, in which yeast was suspended, produced: (a) some diminution of carbohydrate storage, rather varying in amount, the effect increasing with the concentration of the salt (Table V); (b) marked diminution in the amount of fat stored; (c) accumulation of carbonyl compounds in the media to a varying extent.

Similar results were obtained when the acetates of Ca or Mg were added to the glucose solution, less diminution in lipid storage being produced than by CaCl_2 and MgCl_2 ; possibly the acetate had contributed to the fat formation.

The addition of NaCl or Na acetate to the glucose solution markedly inhibited the carbohydrate storage, but unlike Ca^{++} or Mg^{++} , Na^+ only produced a very slight inhibition of lipid storage. Carbonyl compounds were also detected in the medium.

The reaction of the medium did not appear to exercise a very important influence on the storage phenomena. The fat and carbohydrate contents were similar in the alkaline Na acetate-glucose and the acid NaCl-glucose media.

Ca^{++} and Mg^{++} exert a strong inhibitory influence on both fat and carbohydrate synthesis from glucose whereas Na^+ inhibits mainly the carbohydrate synthesis.

The introduction of these three ions especially of Na^+ or Mg^{++} also produced an accumulation of carbonyl compounds in the oxygenated medium. It seems possible, therefore, that this accumulation may have been connected with the diminution of power to store carbohydrate whether by inhibiting its synthesis or promoting its breakdown.

The presence of metallic ions in the solution appears to exercise an important influence on the reactions of the cell which has hitherto not been sufficiently recognized.

The formation of fat from acetates (cf. Table VII)

The influence of K^+ , Na^+ , Mg^{++} and Ca^{++} on the formation of fat from acetate in the absence of sugar is very similar to their effect on the formation of fat from sugar. The average increases in the fat content of a sample of yeast after incubation respectively in oxygenated solutions of *N*/14 acetates and water were:

| Water | Acetate of | | | |
|-------|------------|-------|-------|-------|
| | K | Na | Mg | Ca |
| 41 % | 180 % | 160 % | 118 % | 100 % |

Since the 41 % increase after incubation in water represents the fat formed from reserve carbohydrate, it is clear that fat was formed from acetates and that the inhibitory influence of Ca^{++} and Mg^{++} on the process was considerable. The carbohydrate content of the acetate yeasts was only slightly greater than that of the yeast incubated in water, and since the amount of acetate oxidized was greater in the "run-down" yeast it is probable that the acetate exercised a carbohydrate-sparing action. The level of carbohydrate in the yeast from the acetate solutions and from water was much lower than in the original yeast. We can find no convincing evidence that hydrolysable carbohydrate is formed from acetates. Wieland & Wille [1935] found in a yeast incubated in sodium acetate solution an increase of 6 % of lipid and of 0.03 % hydrolysable carbohydrate

reckoned on the dry weight of yeast, the latter variation being probably within the limits of accuracy of the experiment. This result is in striking contrast to those obtained by incubating yeast in solutions of alcohol when large increases in the storage of hydrolysable carbohydrate took place. It is therefore possible that when fat is formed from alcohol it passes through the stage of storage carbohydrate and that no direct condensation of alcohol to fatty acid takes place.

The conclusion that fatty acid is synthesized from acetate without going through the intermediate stage of hydrolysable carbohydrate is supported by the work of Sonderhoff & Thomas [1937] on the composition of yeast grown in a solution of trideuteracetate. These workers found that under the conditions of their experiment, no exchange of light and heavy hydrogen took place between the trideuteracetate and the water in which it was dissolved. The absorptions of oxygen by yeast were practically equal in acetate or trideuteracetate solutions when tested in the Warburg-Barcroft apparatus. After shaking in the oxygenated trideuteracetate solution, the deuterium contents of the dried yeast and of its lipid and carbohydrate were respectively determined. The acetate was proved to have taken part in the synthesis of lipid since 14.7% of the total lipid hydrogen consisted of deuterium; the percentage of deuterium in the unsaponifiable material was 31.6. Since, in our experiments, incubation in an acetate solution approximately doubled the amount of lipid originally present, and since the original lipid did not contain deuterium, about 30% of the newly formed lipid hydrogen and 63% of that of the newly formed unsaponifiable matter must have come from the trideuteracetate. Our determinations of the constituents of the lipid formed after a brewery yeast had been incubated in Na acetate solutions, showed that the ratio of unsaponifiable to fatty acid was approximately 1 : 3, the same as in the original yeast before incubation.

If a similar relation holds in trideuteracetate solutions, approximately 20% of the hydrogen of the newly formed fatty acid consisted of deuterium derived from the trideuteracetate. This is in agreement with our findings that the lipid is formed by condensation of the acetate molecules.

The yeast carbohydrate (prepared by the method of Sevag & Cattaneo [1935]) contained only 1.6% of its total hydrogen as deuterium.

Although the calculation of the proportions of deuterium in the fatty acid and sterol synthesized from the trideuteracetate can only be a rough approximation, the very marked difference in the proportion of deuterated hydrogen in the unsaponifiable matter (63%) and in the fatty acid (20%) would seem to signify a different manner of condensation of the trideuteracetate. The comparatively small proportion of deuterium remaining in the fatty acid may signify that deuterated water is split off from the condensed molecules which would then be reduced to saturated chains by hydrogen atoms.

In the sterol synthesis, two out of three of its total hydrogen atoms must have been derived from the trideuteracetate.

The influence of phosphates on the formation of fat from acetate and from hexose

The mean lipid content of yeast incubated in a 0.6% solution of Na acetate was found to be unaffected by the addition of Na_2HPO_4 to the medium. Increasing the concentration of the acetate above 0.6% diminished or failed to increase the fat content of the yeast and we could therefore only examine the influence of phosphate in dilute solutions. It is interesting that Katagiri [1926] found that

increasing the concentration of acetic acid in a glucose solution, buffered with Na acetate-acetic acid mixture, exercised an inhibitory effect on fermentation which could not be wholly ascribed to the pH.

When fat is formed from hexose, the effect of phosphate is much more obvious in concentrated than in dilute sugar solutions. Smedley-MacLean & Hoffert [1924, Table III] incubated yeast in oxygenated 4 % fructose solutions, the medium being changed every 48 hr.; their results show the close relationship that exists between the stored carbohydrate and lipid when alkali phosphate is added to the fructose medium.

Consideration of the evidence at present available, seems perhaps to be most consistent with the view that when yeast is incubated in an oxygenated sugar solution, the formation of fat runs parallel with the storage of sugar and phosphate and is probably connected with some stage of the triosephosphate breakdown, pyruvic acid possibly providing the necessary starting material. This process is largely inhibited by the presence of Ca^{++} or Mg^{++} in the medium.

When fat is formed from acetate there is no indication that the hexose-phosphate path is followed; there seems to be a mechanism by which acetate molecules can be directly condensed to form fatty acids; this process is also adversely affected by the presence of Ca^{++} or Mg^{++} . There seems to us to be no indication of the nature of the intermediate stages of this condensation. Wieland & Wille have suggested that acetic acid may be first converted into an "activated" form of succinic acid and that this may pass through oxaloacetic to pyruvic acid. This hypothesis though attractive lacks experimental basis.

Both in the formation of fat from hexose and from acetate, plentiful oxygenation is an essential condition.

SUMMARY

1. When yeast was incubated in an oxygenated solution of any of the following substances, no increase of lipid over that produced on incubation in oxygenated water was observed; acetoin, 2:3-butyleneglycol, methylethyl ketone, sodium salts of citric, succinic, maleic, fumaric, crotonic, laevulic or gluconic acids.

2. Solutions of substances containing two conjugated double bonds, inhibited the lipid increase normally found on incubating in oxygenated water.

3. Addition of Ca^{++} or Mg^{++} to oxygenated glucose solutions in which yeast was incubated markedly diminished the amount of lipid normally stored. Addition of Ca^{++} , Mg^{++} or Na^+ to oxygenated glucose solutions in which yeast was incubated lowered the carbohydrate content whether added as chloride or acetate.

4. When yeast was incubated in oxygenated acetate solutions, Ca^{++} or Mg^{++} lowered the amount of lipid normally formed from the acetate.

5. Addition of phosphate failed to increase the amount of lipid formed from acetate when yeast was incubated in sodium acetate solutions (0.6 %).

6. Addition of K^+ , Na^+ , Ca^{++} or Mg^{++} to a glucose solution in which yeast was incubated, increased the amount of carbonyl substances present in the medium. In experiments in which the acetates of these metals were added to the medium, pyruvic acid and acetaldehyde were identified.

REFERENCES

- Fernbach & Schoen (1913). *C.R. Acad. Sci., Paris*, **157**, 1478.
— — (1914). *C.R. Acad. Sci., Paris*, **158**, 1719.
— — (1920). *C.R. Acad. Sci., Paris*, **170**, 764.
Harden (1917). *Biochem. J.* **11**, 64.
— & Henley (1921). *Biochem. J.* **15**, 312.
Katagiri (1926). *Biochem. J.* **20**, 427.
Kobel & Scheuer (1930). *Biochem. Z.* **229**, 338.
Meyerhof (1918). *Hoppe-Seyl. Z.* **102**, 85.
Neuberg & Kobel (1930). *Biochem. Z.* **229**, 446.
— & Reinfurth (1923). *Biochem. Z.* **143**, 561.
Ohlmeyer & Ochoa (1937). *Biochem. Z.* **293**, 338.
Sevag & Cattaneo (1935). *Liebigs Ann.* **519**, 111.
Smedley-Maclean & Hoffert (1923). *Biochem. J.* **17**, 720.
— — (1924). *Biochem. J.* **18**, 1273.
— — (1926). *Biochem. J.* **20**, 343.
Sonderhoff & Thomas (1937). *Liebigs Ann.* **530**, 195.
Stephenson & Whetham (1922). *Proc. roy. Soc. B*, **93**, 262.
Wieland & Sonderhoff (1932). *Liebigs Ann.* **499**, 213.
— & Wille (1935). *Liebigs Ann.* **515**, 260.